

FY06 Progress Report of the ERSD Project “*Field-Integrated Studies of Long-Term Sustainability of Chromium Bioreduction at Hanford 100H Site*”

1. Research Objectives and Specific Goals

The objectives of the project are to investigate coupled hydraulic, geochemical, and microbial conditions, and to determine the critical biogeochemical parameters necessary to maximize the extent of Cr(VI) bioreduction and minimize Cr(III) reoxidation in groundwater. Specific goals of the project are as follows:

- (1) Field testing and monitoring of Cr(VI) bioreduction in ground water and its transformation into insoluble species of Cr(III) at the Hanford 100H site, to develop the optimal strategy of water sampling for chemical, microbial, stable isotope analyses, and noninvasive geophysical monitoring;
- (2) Bench-scale flow and transport investigations using columns of undisturbed sediments to obtain diffusion and kinetic parameters needed for the development of a numerical model, predictions of Cr(VI) bioreduction, and potential of Cr(III) reoxidation; and
- (3) Development of a multiphase, multi-component 3D reactive transport model and a code, TOUGHREACT-BIO, to predict coupled biogeochemical-hydrological processes associated with bioremediation, and to calibrate and validate the developed code based on the results of bench-scale and field-scale Cr(VI) biostimulation experiments in ground water at the Hanford Site.

(Note that this project is built upon and expands the DOE-EM funded project *Field Investigations of Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) at Hanford 100H*, which was conducted in 2002-2004.)

2. Research Progress and Implications

This report summarizes the results of the work after 2nd year of a 3-year project.

2.1. Field investigations

- The Br-tracer and pumping test was conducted from April 10 until May 2, 2006. During the test, Br concentrations, DO, redox, conductivity, temperature, pH, water levels in the pumping and monitoring wells and the pumping rate were measured.
- Water samples for microbial and geochemical analyses were collected from Wells 699-96-44 and 699-96-45 on November 17, 2005, January 11, 2006, March 29, 2006, and April 17, 20, 24, and May 2, 2006.
- Geophysical survey, including radar and seismic tomography, was conducted on March 30, 2006.
- Drilling and coring of two new boreholes were performed in June-July 2006. Coring included continuous split-spoon sampling of sediments below a depth of 36 ft to the bottom of each well: Well 699-96-42 is ~52 ft—the top of Ringold Upper Mud, and Well 699-96-41 is ~62 ft—terminated within the Ringold mud), (Drilling and coring were conducted according to the Work Plan approved by the Ecology Department of the Washington State.)
- Visual observations of sediments collected during drilling of two new wells showed no apparent changes in the sediments' color or precipitates resulting from the HRC injection.
- New inflatable packers and groundwater samplers were fabricated, tested, and installed in two new wells in September 2006. Two water samplers were installed in Well 699-96-41 and three samplers in Well 699-96-42.

2.2. Geochemical and Microbial Analyses

- A single application of HRC, injected in August 2004, maintained strongly reducing (methanogenic) conditions in a chromate contaminated aquifer for at least 20 months (Figure 1). Some HRC is still likely remained in the formation between the injection and monitoring wells.
- 16S rRNA gene used as biomarker due to a large database and availability of “universal” primers.
- Phylogenetic arrays analysis of water samples demonstrate continued enrichment of iron, sulfate, nitrate reducers, methanogens, methane oxidizers, sulfur oxidizers.

- Preliminary functional array data also show enrichment of nitrate, chromate, iron, and sulfate reducers, sulfur oxidizers, methanogens, and methane oxidizers.
- Combined molecular analyses indicates that chromate reduction is mediated by both direct (chromate reduction) and indirect (Fe^{2+} , H_2S) microbial interaction.
- Analyses of the sediments collected during coring in 2006 show some accumulation of electron acceptors (sulfates, nitrates, and iron) at 47.2-47.7 ft depths near the bottom of the Hanford formation.
- The interpretation of the results of geochemical analyses indicates that the HRC biostimulation has affected differently geochemical processes within the Hanford aquifer:
 - Near the bottom of the Hanford formation: (1) Sulfate concentration dropped to zero approximately 6 months after the HRC injection into Well 699-96-45; (2) Sulfate concentration decreases in the pumping well during pumping, and it rebounds when pumping stops; (3) Carbon isotope ratios of DIC increases in both wells during pumping, reflecting biodegradation of ^{13}C -labeled HRC, and decreases when pumping stops.
 - Near the Hanford aquifer water table: (1) Sulfate drops to a lesser extent than that in the deep intervals near the bottom of the Hanford formation, but exhibits a similar pattern; (2) Nitrate in the pumping well decreases to zero during pumping, but rebounds when pumping stops; (3) Carbon isotope ratios of DIC increase during pumping, but not as much as in the deeper intervals.

2.3. Geophysical Investigations

- The interpretation of the results of seismic and radar surveys confirms that HRC is detectable at a field scale.
- Changes in the radar amplitudes are similar to those from electrical conductivity measurements in boreholes, which are caused by changes in the total dissolved solids (TDS) between the wells, changes in nitrate/sulfate concentrations during pumping or minor mineralogical changes. (These results need further verification using modeling.)
- Changes in the dielectric parameters likely indicate changes in mineralogy and/or reduction in pore space (leading to the formation of gases). (This conclusion needs further verification using experimental and modeling studies.)
- Seismic measurements indicate the effects of HRC injection in the Ringold and Hanford formations, including the formation of gasses and sulfide precipitates.
- Geophysical investigations should be conducted in conjunction with field and laboratory geochemical measurements and modeling, which, in turn, will help reduce non-uniqueness of geophysical signatures.

2.4. Numerical Modeling

- A preliminary model was developed using a 2D (plan view) numerical grid for simulating the Hanford formation aquifer—24 m long, 12 m wide, and 1 m thickness, with the injection Well 699-96-45 at the centre of the model. The grid is radial near the injection and pumping wells, and is rectangular within the rest of the model domain.
- A new 3D grid was created by stacking five 2D plan grids on the top of each other to represent the saturated Hanford formation.
- Preliminary reactive transport modeling included simulations of the HCl injection (as analogue to the lactic acid injection) into the Hanford formation to assess the effects of several minerals—calcite, K-feldspar, albite and quartz.

3. FY 2007 Planned Activities

3.1. Field Investigations

- Use elevation survey data and water level measurements at all 5 wells to define the local groundwater flow direction.
- Continue monthly water level monitoring and water sampling from the monitoring wells.
- Perform a series of field radar and seismic measurements using observations in all 5 wells.

- Conduct a slug interference test in conjunction with the tracer and pumping tests using observations in 4 monitoring wells.
- Consider the need for drilling and completion of a new monitoring well (if needed, obtain a variance from the Ecology Department of the Washington State).
- Consider conducting a new HRC injection at 100-H Site.

3.2. Microbial and Geochemical Analyses

- Microbial and geochemical analyses of sediments from new wells drilled in 2006.
- Use enrichments for microbial isolation of microbial communities using water samples and sediments.

3.3. Numerical Modeling

- Perform a numerical analysis of the results of the tracer and pumping tests conducted in 2004-2006. Develop an updated 3D model of hydrogeological conditions (heterogeneity, saturated hydraulic conductivity, specific yield) to be used as a basis for the numerical chemical transport modeling. Incorporate the effect of the the Ringold Formation and the unsaturated zone.
- Continue the development of a numerical reactive chemical transport model using the specific HRC (glycerol (tri)polylactate) parameters (viscosity, capillary characteristics, thermodynamic data), and the formation mineralogical and geochemical data. In particular, incorporate the geochemical effects of mineralogical phases, Cr and Fe species, thermodynamic and kinetic data.
- Simulate the long-term effect of the HRC injection on Cr(VI) bioremediation and Cr(III) precipitation and sustainability in groundwater.

3.4 Data Analysis and Preparation of Scientific Publications

- Create a project database including the data collected from field observations, field and laboratory experiments, and numerical modeling.
- Continue the analysis of the microbial, geochemical, hydrogeological, geophysical, and modeling studies.
- Write and publish a series of peer reviewed papers.

3.5. Implementation

- The results of this project will be used to provide design parameters for a treatability study of bioremediation of 100-D to improve the effectiveness of the ISRM barrier (project supported by EM-22).
- Collaboration with the Oregon State University (Jack Istok) in predictions of the effect of lactate addition on the microbial community and geochemistry at the 100H area.

IV. Information Access

- The project website is at <http://www-esd.lbl.gov/ERT/hanford100h/>
- A special FTP site with the access to all data collected during the project and publications has just been created.
- FY2006 List Publications and Presentations
 1. Conrad, M.E., S.E. Borglin, E. Brodie, K.N. Woods, B.A. Faybishenko, T.C. Hazen, P.E. Long, and A. Willett, Quantification of enhanced bioreduction of Cr(VI) using $\square^{13}\text{C}$ -PLFA monitoring coupled with advanced microbial techniques. Abstract No. 97274 published in GSA Abstracts with Programs, Vol. 37, No. 7, 2005.
 2. Hubbard, S., J. Peterson, J. Chen, K. Williams, M. Conrad, B. Faybishenko, A. Willet, P. Long, and T. C. Hazen. Geophysical Monitoring of Cr(VI) bioreduction at the Hanford 100H Site. December 2005, San Francisco, CA. AGU Fall Meeting.
 3. Hazen, T. C. Invited. Integrated Omics in Systems Biology: The New Frontier for Environmental Biotechnology, Ecology and Evolution. September 2006, Mayaguez, Puerto Rico. 3rd Latin American and Caribbean Biotechnology Conference.
 4. Hazen, T. C. Invited. Long-Term Chromium Bio-Immobilization at the Hanford 100H Site: Geochemical and Microbiological Response to Slow Release Electron Donor. September

- 2006, Mayaguez, Puerto Rico. Department of Biology, University of Puerto Rico at Mayaguez.
5. Hazen, T. C. Invited Keynote Speaker. Integrated Omics in Systems Biology: The New Frontier for Environmental Biotechnology, Ecology and Evolution. September 2006, Humacao, Puerto Rico. 2da Actividad Regional de Biotecnologia-UPR-Humacao "Biotecnologia en al Dario vivir: mejorando nuestra calidad de vida".
 6. Hazen*, T. C. and A. P. Arkin. Contributed. Integration of Omics, Bioinformatics, and Biogeochemistry: The New Frontier for Environmental Biotechnology. August 2006, Vienna, Austria. 11th International Symposium on Microbial Ecology.
 7. Brodie*, E. L., T. C. Hazen, B. Faybishenko, D. Joyner, S. E. Borglin, R. Chakraborty, E. Shapland, M. Conrad, T. Tokunaga, J. Wan, S. Hubbard, K. Williams, M. Firestone, G. L. Andersen, T. DeSantis, P. E. Long, D. R. Newcomer, and S. Koenigsberg. Contributed. High Density 16S rRNA Microarray Analysis of Long-Term Chromium Bio-immobilization. August 2006, Vienna, Austria. 11th International Symposium on Microbial Ecology.
 8. Hazen, T. C. Invited. Integrated Omics in Systems Biology: The New Frontier for Environmental Biotechnology, Ecology and Evolution. August 2006, Beijing, China. Institute of Microbiology, Chinese Academy of Sciences.
 9. Hazen, T. C. Invited. Long-Term Chromium Bio-Immobilization at the Hanford 100H Site: Geochemical and Microbiological Response to Slow Release Electron Donor. August 2006, Beijing, China. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.
 10. Hazen, T. C. Invited. Integrated Omics in Systems Biology: The New Frontier for Environmental Biotechnology, Ecology and Evolution. August 2006, Changsha, China. Central South University
 11. Hazen, T. C. Invited. Advances in Field Research on Bioremediation of Metals and Radionuclides. August 2006, Changsha, China. Central South University.
 12. Long*, P. E., D. R. Newcomer, C. T. Resch, K. Cantrell, B. Faybishenko, T. C. Hazen, E. Brodie, D. Joyner, S. Borglin, J. Hanlon, M. Conrad, T. Tokunaga, J. Wan, S. Hubbard, K. Williams, J. Peterson, M. Firestone, G. Andersen, T. DeSantis, A. Willett, and S. Koenigsberg. Contributed. Evaluation of the Effectiveness of Cr(VI) Biostimulation in Groundwater at Hanford 100H Site. May 2006, Baltimore, MD. Spring meeting of American Geophysical Union.
 13. Faybishenko*, B., T. C. Hazen, E. Brodie, D. Joyner, S. Borglin, J. Hanlon, M. Conrad, T. Tokunaga, J. Wan, S. Hubbard, K. Williams, J. Peterson, M. Firestone, G. Andersen, T. DeSantis, P. E. Long, D. R. Newcomer, C. T. Resch, A. Willett, and S. Koenigsberg. Contributed. Tracer Tests and Field Monitoring of In situ Cr(VI) Bioreduction at the Hanford 100H Site. May 2006, Baltimore, MD. Spring meeting of American Geophysical Union.
 14. Brodie*, E., and T. C. Hazen. Contributed. Integration of the Omics, Bioinformatics, and Biogeochemistry: The New Frontier for Environmental Biotechnology. May 2006, Monterey, CA. The Fifth International Conference on Remediation of Chlorinated and Recalcitrant Compounds.
 15. Tabak, H. H., and T. C. Hazen*. Contributed. Advances in Bioremediation of Soils and Sediments Polluted with Metals and Radionuclides: 2. Field Research on Bioremediation of Metals and Radionuclides. May 2006, Monterey, CA. The Fifth International Conference on Remediation of Chlorinated and Recalcitrant Compounds.
 16. Hazen*, T. C., B. Faybishenko, E. Brodie, D. Joyner, S. E. Borglin, R. Chakraborty, M. Conrad, T. Tokunaga, J. Wan, S. Hubbard, K. Williams, J. Peterson, M. Firestone, G. Andersen, T. DeSantis, P. E. Long, D. R. Newcomer, A. Willett, and S. Koenigsberg. Contributed. Long-Term Chromium Bio-Immobilization at the Hanford 100H Site: Geochemical and Microbiological Response to Slow Release Electron Donor. May 2006, Orlando, FL. American Society for Microbiology Annual Meeting.
 17. Borglin*, S. E., M. E. Conrad, E. Brodie, K. N. Woods, B. Faybishenko, D. Joyner, and T. C. Hazen. Contributed. Assessment of Bioreduction of Cr(VI) Using ¹³C-PLFA analysis. May 2006, Orlando, FL. American Society for Microbiology Annual Meeting.

18. Hazen, T. C. Invited. Integrated Omics in Systems Biology: The New Frontier for Environmental Biotechnology, Ecology and Evolution. April 2006, Miami, OH. Orton K. Stark General Lecture, Miami University.
19. Hazen, T. C. Invited. Bioremediation: Cleaning up using Nature's Natural Cleansing Capacity. April 2006, Miami, OH. Orton K. Stark General Lecture, Miami University.
20. Hazen, T. C. Invited. Stimulating the Microbial Reduction of Chromium. April 2006, Warrenton, VA. DOE ERSP annual workshop.
21. Hazen*, T. C., B. Faybishenko, E. Brodie, D. Joyner, S. Borglin, R. Chakraborty, M. Conrad, T. K. Tokunaga, J. Wan, S. Hubbard, K. Williams, J. Peterson, M. Firestone, G. Andersen, T. DeSantis, P. E. Long, D. R. Newcomer, A. Willett, and S. Koenigsberg. Invited. Long-Term Chromium Bio-Immobilization at the Hanford 100H Site: Geochemical and Microbiological Response to Slow Release Electron Donor. April 2006, Warrenton, VA. DOE ERSP annual workshop.
22. Hazen, T. C. Invited. Omics and Bioremediation Breakout Session. April 2006, Warrenton, VA. DOE ERSP annual workshop.
23. Hazen, T. C. Invited. Engineered Microbial Systems. March 2006, Atlanta, GA. Georgia Tech Environmental Systems Microbiological Symposium.
24. Hazen*, T. C. and H. H. Tabak. Invited. Field Research on Bioremediation of Metal Contamination in Soils and Sediments. March 2006, San Diego, CA. The Sixteenth Annual AEHS Meeting and West Coast Conference on Soils, Sediments and Water.
25. Hazen, T. C. Moderator. Bioremediation Strategies for Contaminated Sediments. March 2006, San Diego, CA. The Sixteenth Annual AEHS Meeting and West Coast Conference on Soils, Sediments and Water.
26. Hazen, T. C. Invited. Tutorial on Systems Biology and Bioremediation. January 2006, Winston-Salem, NC. Wake Forest University
27. Hazen, T. C. Invited. Integrated Omics in Systems Biology: The New Frontier for Environmental Biotechnology, Ecology and Evolution. January 2006, Winston-Salem, NC. Wake Forest University.
28. Hubbard*, S., J. Peterson, J. Chen, K. Williams, M. Conrad, B. Faybishenko, A. Willet, P. Long, and T. C. Hazen. Contributed. Geophysical Monitoring of Cr(VI) bioreduction at the Hanford 100H Site. December 2005, San Francisco, CA. Annual Meeting of the American Geophysical Union.
29. Long, P. E., Terry Hazen, Boris Faybishenko, et al., Field Investigations of Lactate-Stimulated Bioreduction of Cr(VI) to Cr(III) at Hanford 100-H Area. Invited presentation to the Hanford Site Unit Manager Meeting, Richland, Washington April 20, 2006.

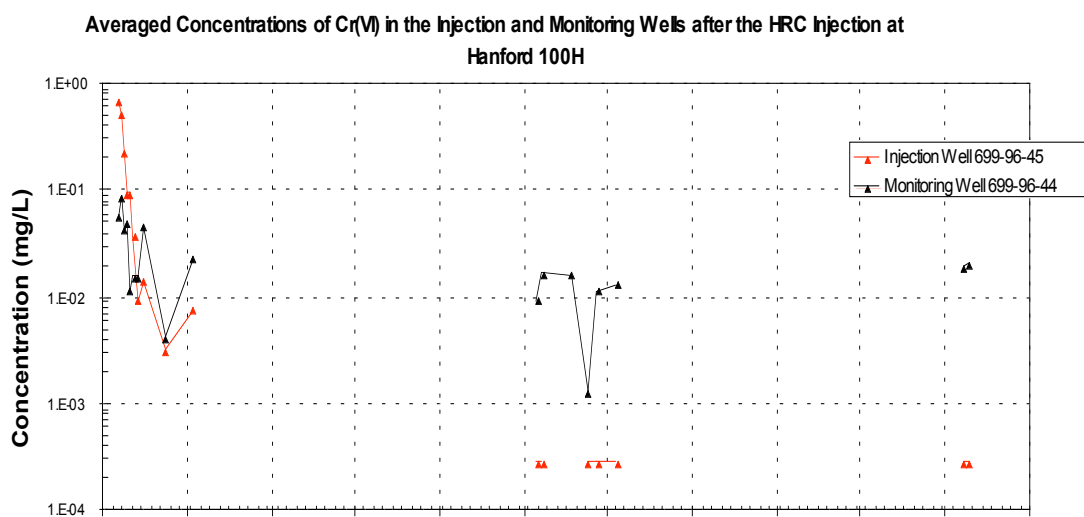


Figure 1. Changes in averaged concentrations of Cr(VI) in water samples collected from the HRC injection and monitoring wells. (Note: HRC was injected on August 3, 2006)

Pacific Northwest National Laboratory's Role in ERSD Project "*Field-Integrated Studies of Long-Term Sustainability of Chromium Bioreduction at Hanford 100H Site*":

Pacific Northwest National Laboratory (PNNL) coordinates and supports all field activities for this project. PNNL also performs field sample collection and shipping, including collection and analysis of field geochemical parameters. PNNL arranges all drilling and site services activities for the project through Hanford Site Contractors. PNNL staff participate in data analysis and interpretation of results, including presenting results to Hanford DOE-EM personnel and their contractors. PNNL's activities in this arena resulted in a successful bioremediation application project for the Hanford 100 D Area.

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